Factors affecting degradation rates of five triazole fungicides in two soil types: 1. Laboratory incubations

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Abstract: Triazole fungicides are now widely used commercially and several are known to be persistent in soil. The degradation rates of five such fungicides were measured in laboratory tests with two soils over 720 days, with analysis of soil extracts by high-pressure liquid chromatography. Behaviour in a sandy loam and a clay loam were similar, and incubation of the compounds either singly or in admixture did not influence loss rates except for those of flutriafol which were lower in the latter. Triadimefon was quite rapidly reduced to triadimenol, though traces of the former were always found, indicating a possible redox equilibrium. Flutriafol, epoxiconazole and triadimenol (derived from triadimefon) were very persistent, breakdown following first-order kinetics with half-lives greater than two years at 10 °C and 80% field capacity. Propiconazole was moderately persistent, with a half-life of about 200 days under these conditions. Degradation rates increased about 3-fold as the temperature was increased from 5 to 18 °C, though decreasing soil moisture to 60% field capacity only slightly slowed degradation. The rate constants obtained are used in a companion paper describing field studies on these two soils to compare laboratory-measured degradation rates with losses in the field following commercial sprays.

Keywords: flutriafol; epoxiconazole; propiconazole; triadimefon; triadimenol; triazole fungicides; persistence in soil

1 INTRODUCTION

Several classes of fungicide act by inhibiting sterol biosynthesis.[1] One such class is typified by having two or three aryl rings attached to a central carbon or small aliphatic core, an early example being triarimol reported in 1969. These compounds require a heterocyclic ring for activity; initially a pyrimidine was used but, by the 1970s, the 1,2,4-triazole ring was found to give improved activity. Such compounds are loosely called 'triazole fungicides', and are typically applied as foliar sprays to growing crops, or sometimes as seed treatments.

These triazole fungicides are now widely used, and a possible cause for concern is their long persistence in soil. Most information on persistence is derived from registration documents, with relatively few studies appearing in the open literature. Flutriafol (then coded as PP450) was very persistent in laboratory tests in a clay loam soil at 15 °C though simple substituted benzyltriazoles were rapidly degraded by micro-organisms, indicating that the triazole moiety does not necessarily confer stability.[2] Another example is triadimenol, derived by fairly rapid reduction of triadimefon in soil, for which Bromilow *et al*[3] observed half-lives of about one year at 15 °C for both diastereoisomers in laboratory incubations. In contrast, difenoconazole was reported to have a DT_{50} of only 33–54 days in laboratory tests at 30 °C, with pretreatment and addition of leaf litter both reducing persistence.[4]

However, largely anecdotal reports suggest that persistence in the field may often be less than that predicted from laboratory tests. Bromilow *et al*[3] could not detect any residues of triadimenol in soil after 12 years of consecutive annual applications of triadimefon to soil at 0.25 kg ha^{-1}, despite its persistence in this same soil in the tests outlined above.

This paper examines the influence of temperature and moisture on the breakdown of five triazole fungicides (flutriafol, epoxiconazole, propiconazole, triadimefon and triadimenol) in two soils of contrasting type. These studies complement parallel field studies[5] on these same compounds and so allow comparisons between observed behaviour in the field and predictions thereof based on these laboratory tests.

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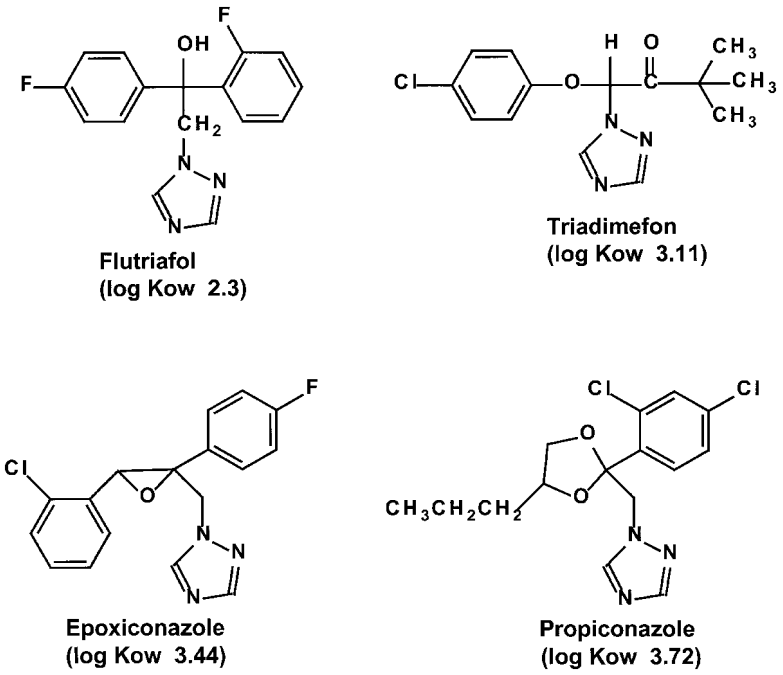

Figure 1. Chemical structures of the four triazole fungicides and their lipophilicity as 1-octanol/water partition coefficients (K_{ow}).

2 MATERIALS AND METHODS

2.1 Triazole fungicides

Compounds (Fig 1) were supplied by the manufacturers, propiconazole being technical grade (90.3%) and the others analytical grade (>98%). Epoxiconazole is used commercially as a single diastereoisomer, whereas triadimenol (generated by reduction of triadimefon in soil to the secondary alcohol, and having log K_{ow} 3.2) and propiconazole each comprises a pair of diastereoisomers.

2.2 Soils

Soils were collected from the topsoils of Foster's Corner, Rothamsted, Hertfordshire and Road Piece, Woburn, Bedfordshire and kept as sampled at 10 °C for a few days as necessary until use. The soil properties are given in Table 1.

2.3 Incubations of triazole fungicides

Soils were sieved to 4 mm without drying. Two series of incubations were set up, either utilising four fungicides (flutriafol, epoxiconazole, propiconazole and triadimefon) in admixture, and under various incubation conditions of soil temperature and moisture, or each of these compounds individually at just one set of ambient conditions. These latter individual incubations served to check that there were no interactions between the fungicides when incubated together that influenced persistence. The application rate was 1.0 μg g^{-1} moist soil for each compound, this being equivalent to a typical maximum annual commercial application of 500 g ha^{-1} distributed within the top 5 cm of soil (assuming a soil density of 1 g ml^{-1}).

The fungicides (either together or individually) were dissolved in acetone (12 ml, to give a concentration of 100 μg ml^{-1}) and distributed over the surface of the sieved moist soil (1.2 kg) spread on aluminium foil, and the acetone allowed to evaporate. Soils were then made up to the required moisture content (60, 80 or 100% field capacity), thoroughly mixed by rolling for 10 min in a 50-litre drum and placed as a 10-cm layer in 3-litre glass screw-capped jars (11 × 12 × 24 cm high) which were kept in the dark. Temperature was maintained at 10 °C for these three moisture contents, with additional incubations for the 80% field capacity (FC) at 5°, 15° and 18 °C for the fungicide mixtures. Soils receiving the single fungicide were incubated at 80% FC and 10 °C only. The incubation jars were aerated weekly and the soil-moisture content maintained periodically as necessary. Samples of soil (20 g), in triplicate at day 0 and in duplicate thereafter, were taken for analysis at intervals of at least once a month over a two-year period.

Table 1. Properties of the soils

		Size distribution (%)	
Soil fraction (μm)[a] or property		*Rothamsted clay loam*	*Woburn sandy loam*
Sand	600–2000	2.0	1.9
	212–600	3.4	41.7
	106–212	4.5	34.2
	63–106	1.7	1.5
Silt	2–63	62.5	9.6
Clay	<2	25.9	11.2
Organic carbon (%)		1.4	0.9
pH[b]		7.2	6.7
Field capacity (% w/w)		20.0	13.9

[a] Measured by the UK Soil Survey and Land Research Centre.
[b] Measured in a slurry of soil (10 g) + aqueous calcium chloride (0.01 M; 25 ml).

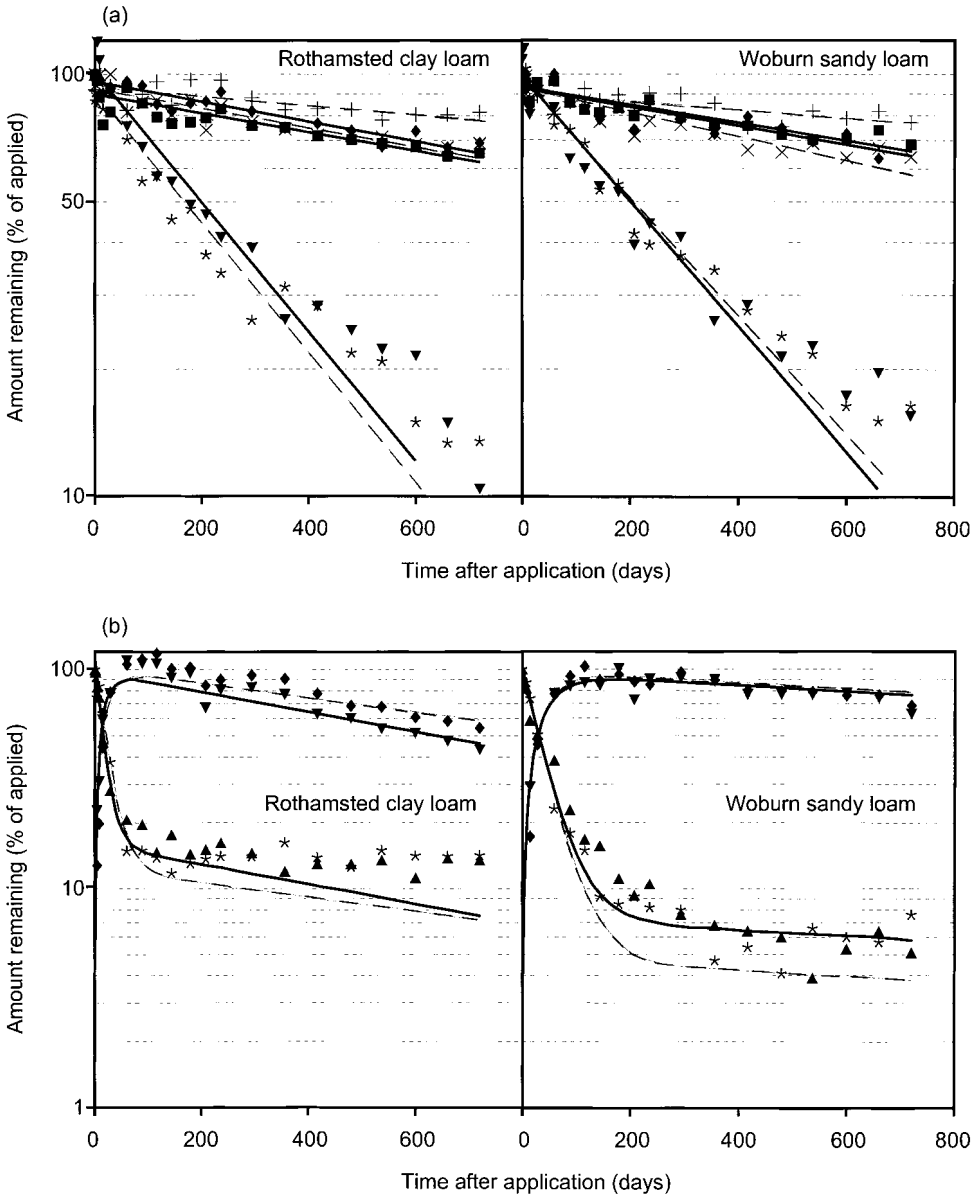

Figure 2. Breakdown in soil at 10 °C and 80% field capacity of compounds applied (—) individually or (---) in admixture (a) (■; +) flutriafol, (●; ×) epoxiconazole and (▼; *) propiconazole (b) (▲; *) triadimefon and (▼; ◆) derived triadimenol.

2.4 Measurement of fungicides in the soil samples

Each soil sample (20 g) was extracted by orbital shaking in a 500-ml glass stoppered jar with methanol (100 ml) for 4 h. After allowing to settle, an aliquot (50 ml) of supernatant solution was rotary evaporated to dryness at a bath temperature not exceeding 40 °C. The residue was dissolved in hexane + acetone (5 + 1, by volume, 2.0 ml) and placed onto a Waters Sep-Pak silica cartridge prewashed with the same solvent (2.0 ml). The flask was rinsed onto the cartridge with a further aliquot (1.0 ml), and the eluate discarded. The fungicides were eluted with hexane + acetone (1 + 1, by volume, 5.0 ml). This fraction was evaporated to dryness as above, and dissolved in methanol + water (70 + 30, by volume, 1.0 ml) with brief ultrasonication.

Analysis was by high-pressure liquid chromatography (HPLC) on a Pye Unicam 4000 series instrument fitted with a 20-µl injection loop. The column was 20 cm × 4.6 mm ID packed with 10 µm LichroCART RP-18 protected by a guard column, and the mobile phase was methanol + water (70 + 30, by volume) at a flow rate of 1.5 ml min^{-1}. Detection was by UV at 215 nm. Under these conditions, all five compounds could be separated over 15 min; the diastereoisomers of triadimenol and of propiconazole were not clearly resolved, and each pair was assessed as a single compound. Typical retention times were 3.25, 5.75, 6.62, 8.00 and 11.75 min for flutriafol, triadimefon, triadimenol, epoxiconazole and propiconazole, respectively. Recoveries of the fungicides added to the two soils ranged between 89.1 and 110.4%; all results were corrected for the appropriate recovery factor. To confirm the persistence of small amounts of triadimefon, additional HPLC was done using acetonitrile + water (50 + 50, by volume) as the mobile phase, in

Table 2. Effect of soil temperature at 80% field capacity on breakdown rates of triazole fungicides

	Half-life, $t_{1/2}$ (days)							
	Rothamsted clay loam				Woburn sandy loam			
Fungicide	5°C	10°C[a]	15°C	18°C	5°C	10°C[a]	15°C	18°C
Flutriafol	2310	2888 (1359)	2038	1650	3850	3013 (1444)	1575	1444
Epoxiconazole	1507	1332 (1283)	1100	1004	1540	1066 (1359)	815	737
Propiconazole	408	195 (196)	135	113	499	215 (203)	117	105
Triadimefon	35.7	16.9 (12.8)	9.8	6.8	58.8	29 (29.5)	17.4	13.4
Triadimenol[b]	2003	826 (573)	447	363	6930	2005 (2026)	805	624

[a] Numbers in parentheses are the half-lives for the corresponding individual incubation at 10°C and 80% FC.
[b] Derived from application of triadimefon.

which the order of elution of triadimefon and triadimenol was reversed and the latter was resolved into its diastereoisomers.

2.5 Derivation of rate constants

Rate constants were fitted in models using least squares (Maximum Likelihood Program: MLP),[6] which assumed first-order kinetics. The reduction of triadimefon to triadimenol appeared to reach a redox equilibrium, which accounts for the persistence of small amounts of the former.

3 RESULTS AND DISCUSSION

All degradation processes fitted first-order kinetics, indicating that there were no appreciable differences in the behaviours of the diastereoisomers (triadimenol and propiconazole) which were treated as single compounds in the kinetic analysis. This is in agreement with previous detailed observations of the similar breakdown rates in Rothamsted soils of the diastereoisomers of triadimenol.[3]

Triadimefon and triadimenol were broken down about twice as quickly in the Rothamsted clay loam as in the Woburn sandy loam, but otherwise only small differences in breakdown behaviour were seen between the two soils. Degradation rates of these fungicides were mostly similar in the incubations at 10 °C and 80% FC whether applications to soil were of a single compound or of the four compounds together (Fig 2, semi-logarithmic plots). Flutriafol was an apparent exception in that incubation in admixture doubled the half-life in both soils, though it was already persistent even when incubated individually. Thus there appeared to be no important interactions between the compounds in these tests, and the decision, on logistical grounds, to apply the four compounds together in the field, and in the detailed laboratory incubations, has not led to atypical breakdown behaviour.

Reduction of triadimefon to triadimenol was fairly rapid in both soils, appearing to reach an equilibrium with about 14% and 5% of triadimefon remaining in the Rothamsted and Woburn soils, respectively. Reduction was slightly favoured in the Woburn soil by both higher temperatures and soil moisture contents, but was little influenced by conditions in the Rothamsted soil. Extracts of blank soils did not cause any interference in the HPLC at the retention time of triadimefon, and examination of the soil extracts from 660 days by HPLC using the second mobile-phase system confirmed both the presence and amounts of triadimefon in the samples. Persistence of triadimefon at these levels was not observed in the previous similar study by Bromilow *et al*,[3] in which it was not detected beyond 36 days; however, small amounts of persisting triadimefon were also found in the current complementary field studies,[5] indicating that traces of triadimefon may resist complete reduction in soil, either by being protected by sorption to soil and/or by

Table 3. Effect of soil moisture content (% field capacity) at 10 °C on breakdown rates of triazole fungicides

	Half-life, $t_{1/2}$ (days)					
	Rothamsted clay loam			Woburn sandy loam		
Fungicide	60% FC	80% FC	100% FC	60% FC	80% FC	100% FC
Flutriafol	2567	2888	2235	5775	3013	3013
Epoxiconazole	1540	1332	1283	1444	1066	1136
Propiconazole	336	195	175	277	215	237
Triadimefon	21.2	16.9	14.5	35.9	29	26.2
Triadimenol[a]	1003	826	772	3501	2005	1805

[a] Derived from application of triadimefon.

Table 4. Activation energies for breakdown of triazole fungicides in two soils

	Activation energy (J mol^{-1})	
Fungicide	Rothamsted clay loam	Woburn sandy loam
Flutriafol	(8134)[a]	23550
Epoxiconazole	9129	16370
Propiconazole	27980	35260
Triadimefon	36180	32540
Triadimenol[b]	38020	39420

[a] Unreliable due to low correlation coefficient.
[b] For degradation other than oxidation back to triadimefon.

reaching a redox equilibrium with triadimenol. Subsequent breakdown of triadimenol was very slow under all conditions, as was also observed for flutriafol and epoxiconazole; these three compounds had half-lives of over two years at 10 °C and 80% FC.

Propiconazole was somewhat more rapidly broken down, though even for this compound the half-life at 10 °C and 80% FC was about 200 days. Faster breakdown at the higher temperatures (Table 2) and slight slowing in the driest soil tested (60% FC) could be clearly seen (Table 3) for propiconazole. This also appeared to occur for the other fungicides, although these effects were less discernible due to the more limited breakdown. Such observed persistence is in agreement with that reported previously for flutriafol[2] and triadimenol,[3] the latter being apparently slightly more persistent in this study with $t_{1/2}$ of 447 days compared to about 350 days previously, both measured at 15 °C and in similar Rothamsted clay loam soils. Though sorption to soil must play a part in slowing breakdown, it is of interest to note that the most polar (and hence least sorbed)[5] fungicide flutriafol (Fig 1) was the most recalcitrant, whilst the most lipophilic, propiconazole, underwent the most rapid true degradation.

Standard errors on the rate constants were typically ± 3 to 10% for breakdown of propiconazole and triadimefon, but up to $\pm 25\%$ or more for the much slower breakdown of epoxiconazole, flutriafol and triadimenol, especially at the lower temperatures. This variability may be the cause of apparent anomalies, such as the breakdown of flutriafol in the Rothamsted soil at 80% FC appearing to be slower at 10 ° than 5 °C. However, most of the half-lives were ranked consistently with temperature, and activation energies were estimated (Table 4) using the Arrhenius equation. The fit was generally excellent with $r^2 > 0.95$, except for the slow breakdown of flutriafol in the Rothamsted soil, where the estimate of activation energy was unreliable with $r^2 < 0.5$. Decreasing soil moisture from field capacity to 80% FC had little effect on half-life (Table 3), though at 60% FC degradation was generally somewhat slower. Though the measured breakdown rates would not allow accurate extrapolation to colder or drier soils, degradation would be so slow under these unfavourable conditions that these uncertainties would not present a weakness in modelling behaviour in the complementary field trials.

These incubations were continued over 720 days in order to obtain measurable breakdown. However, registration authorities often declare that soil may lose its activity after 120 days in such laboratory tests, although fields may be left fallow for over 200 days over winter prior to the planting of spring crops. In our tests, microbial degradation processes are thought to dominate, as substituted l-benzyltriazoles are degraded microbially,[2] and also these triazole fungicides have no labile functionality amenable to chemical breakdown processes. That first-order kinetics generally prevailed in our tests over 720 days indicates that the soil activity did not markedly change, indicating that the suggested 120-day 'cut off' may be overcautious. Only the degradation of propiconazole slowed with time, but this effect was not appreciable until well into the third half-life.

4 CONCLUSIONS

These studies confirm the slow breakdown of four examples of triazole fungicides in two differing soils. Degradation followed essentially first-order kinetics over 720 days of incubation, and was little sensitive to soil moisture content in the range tested, but showed the typical increase in breakdown rate with increasing temperatures from 5 to 18 °C. Flutriafol, epoxiconazole and triadimenol were persistent, with $t_{1/2} >$ two years at 10 °C and 80% FC, whilst propiconazole was somewhat less persistent with $t_{1/2} \sim 200$ days. Triadimefon was more rapidly transformed, though this reduction to the persistent triadimenol did not go to completion, as traces of the former were still present at 720 days.

The degradation rates observed for flutriafol, epoxiconazole and triadimenol in both the clay loam and sandy loam soil are so low that degradation in the field at winter temperatures, or in topsoil during dry periods in the summer, would be expected to be very slow. A companion paper[5] uses these rate constants, together with climate data for the field experiments run in conjunction with these laboratory tests, to identify any further processes that may be involved in dissipation in the field. This will assist in assessing the usefulness of registration data generated using laboratory degradation tests for understanding and predicting the field behaviour of these often persistent triazole fungicides.

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